

**Investigating the Oxidoreductase  
Activity of Members of the Chloride  
Intracellular Ion Channel Protein Family**

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*A thesis submitted in fulfilment of the requirements for  
the degree of Doctor of Philosophy*

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## **Certificate of Original Authorship**

I, Hala Mishaal Ali, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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## *List of Abbreviations*

|                   |   |
|-------------------|---|
| AsA               | Ascorbic Acid   |
| Å                 | Angstrom  |
| Arg               | Arginine amino acid                                       |
| Asn               | Asparagine amino acid                                     |
| ATCC              | American Type Culture Collection                          |
| BCA               | Bicinchoninic acid assay                                  |
| BSA               | bovine serum albumin                                      |
| °C                | centigrade  |
| CaCCs             | Calcium activated Chloride channel                        |
| CaCl <sub>2</sub> | calcium chloride  |
| CAFs              | Cancer-Associated Fibroblast secretome                    |
| Car               | Carbenicillin   |
| CDCs              | Cholesterol-Dependent Cytolysins                          |
| C-domain          | Carboxy terminal domain                                   |
| cDNA              | complementary Deoxyribonucleic acid                       |
| CFTR              | Cystic fibrosis transmembrane conductance<br>regulator    |
| CHO-k1            | Chinese Hamster Ovary cells                               |
| Chol              | Cholesterol   |
| CIC               | Chloride Ion Channel                                      |
| CLIC              | Chloride Intracellular Ion Channel                        |
| Cl <sup>-</sup>   | Chloride ion  |
| CLIC (WT)         | Chloride Intracellular Ion Channel protein<br>(wild type) |
| Cys               | Cysteine  |
| CV                | column volumes  |
| COPD              | Chronic obstructive pulmonary disease                     |
| Da                | Dalton  |
| DDT               | Dithiotheritol  |
| DHA               | Dehydroascorbate  |

|                               |  |
|-------------------------------|--|
| DHAR                          | Dehydroascorbic acid reductase                     |
| DMSO                          | Dimethyl sulfoxide                                 |
| Dm CLIC                       | <i>Drosophila melanogaster</i> CLIC protein        |
| DNA                           | Deoxyribonucleotides acid                          |
| DIDS                          | 4, 4'Di isothiocyano-2, 2'-stilbenedisulfonic acid |
| ECM                           | extracellular matrix                               |
| EDTA                          | ethylene diamine tetra acetic acid                 |
| <i>E-coli</i>                 | <i>Escherichia coli</i>                            |
| EPR                           | Electron Paramagnetic Resonance                    |
| ER                            | Endoplasmic Reticulum                              |
| ERK7                          | Extracellular signal-regulated kinase 7            |
| EXC                           | Excretory Canal abnormality                        |
| EXL                           | EXC4-like  |
| FRET                          | Fluorescence Resonance Energy Transfer             |
| 3D                            | three dimensions                                   |
| GABA                          | Gamma-Amino Butyric Acid                           |
| Glu                           | Glutamic amino acid                                |
| Grx                           | Glutaredoxin                                       |
| GR                            | glutathione reductase                              |
| GSH                           | Reduced glutathione                                |
| G-site                        | Glutathione binding site                           |
| GST                           | Glutathione S-transferase                          |
| HEDS                          | 2-hydroxyethyl disulphide                          |
| His                           | Histidine  |
| H <sub>2</sub> O <sub>2</sub> | hydrogen peroxide                                  |
| HEPES                         | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| H <sub>2</sub> O              | water  |
| IAA                           | Indanyloxyacetic acid                              |
| IPTG                          | Isopropyl-thio-β-D-galactopyranoside               |
| Kan                           | Kanamycin  |
| KCl                           | potassium chloride                                 |

|                                  |  |
|----------------------------------|--|
| KH <sub>2</sub> PO <sub>4</sub>  | potassium dihydrogenphosphate                          |
| K <sub>2</sub> HPO <sub>4</sub>  | dipotassium hydrogenphosphate                          |
| LB                               | Luria broth  |
| Lys                              | Lysine amino acid                                      |
| MAP kinase                       | Mitogen Activated Protein kinase                       |
| μm                               | micro molar  |
| NAD <sup>+</sup>                 | nicotinamide adenine dinucleotide                      |
| NaOH                             | sodium hydroxide                                       |
| NaCl                             | sodium chloride  |
| NADPH                            | reduced nicotinamide adenine dinucleotide<br>phosphate |
| Na <sub>2</sub> HPO <sub>4</sub> | disodium hydrogen phosphate                            |
| NCC27                            | Nuclear Chloride Channel protein-27 KDa                |
| NEB                              | New England Biolabs                                    |
| Ni <sup>2+</sup> ions            | nickel ions  |
| NTA                              | nitrilotriacetic acid matrix                           |
| N-domain                         | Amino terminal domain                                  |
| NMR                              | Nuclear Magnetic Resonance                             |
| O.D.                             | optical density  |
| OH•                              | hydroxyl radical                                       |
| ORF                              | open reading frame                                     |
| P64                              | bovine chloride channel protein                        |
| PFT                              | Pore Forming Toxin                                     |
| PBS buffer                       | Phosphate-buffered saline                              |
| PICOT                            | protein kinase C interacting cousin of<br>thioredoxin  |
| Phe                              | Phenylalanine amino acid                               |
| PTMD                             | Putative Transmembrane Domain                          |
| Pro                              | Proline amino acid                                     |
| PLB                              | Planner Lipid Bilayer                                  |
| PVDF                             | polyvinylidene fluoride membrane                       |
| PTM                              | post-translational modification                        |
| RT                               | room temperature                                       |

|            |  |
|------------|--|
| RT-PCR     | Real-time quantitative Polymerase Chain Reaction           |
| RNR        | ribonucleotide reductase                                   |
| RyR        | Ryanodine receptor   |
| ROS        | Reactive Oxygen Species                                    |
| SDS-PAGE   | Sodium Dodecyl Sulfate -Polyacrylamide                     |
| Ser        | Serine amino acid  |
| SEC        | Size Exclusion Chromatography                              |
| TBE Buffer | tric,boric acid, ethylene diamine tetra acetic acid buffer |
| TCEP       | tris-2-carboxyethyl-phosphine                              |
| TEMED      | NNNN'-tetramethylethylenediamine                           |
| TGM2       | transglutaminase-2   |
| Trp 35     | Tryptophan 35 in CLIC1                                     |
| TMD        | Transmembrane Domain                                       |
| Trx        | Thioredoxin  |
| TrxR       | thioredoxin reductase                                      |
| UV         | Ultraviolet  |
| Val        | Valine amino acid  |
| WCLs       | Whole Cell Lysates   |
| X          | any amino acid   |

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## **Abstract**

The chloride intracellular ion channel (CLICs) proteins are atypical anion selective channel proteins, as they are principally soluble proteins, with some members now known to also demonstrate enzymatic activity. Structural studies demonstrate that the CLIC proteins share strong structural homology with members of the glutathione-S-transferase superfamily of enzymes, in particular the omega glutathione-S-transferase (GST- $\Omega$ ) members.

The discovery that the CLIC proteins have the functional ability to act as glutathione dependent oxidoreductase enzymes, also suggested a role for them as cell protective proteins and antioxidants. Therefore, this PhD project aimed to further define the functional activity of the CLIC proteins, in particular CLIC3 as one of more recently identified members.

The principal findings of this PhD project include demonstrating for the first time that CLIC3 has glutathione dependant oxidoreductase activity via its dithiol active enzymatic site. In this study, we directly contributed to the finding that the extracellular activity of transglutaminase-2 (TGM2) is regulated by CLIC3's oxidoreductase activity, and their interaction was dependent upon the redox environment. Furthermore, our *in vitro* studies of CLIC3, were key in helping demonstrate a critical role for secreted CLIC3 in cancer metastasis and tumour cell invasiveness.

To further investigate this oxidoreductase activity of the CLIC proteins we used a bacterial cell model to probe their ability to protect cells against oxidative assault. Recombinant CLIC proteins were expressed in bacterial *E. coli* cells, followed by their exposure to the oxidising agent hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Expression of CLIC1 by the *E. coli* cells was found to provide increased tolerance of up to 5mM H<sub>2</sub>O<sub>2</sub>, while CLIC3

afforded some protection, with no difference seen for cells expressing CLIC4. This work for the first time demonstrates CLIC1 protein acting in antioxidant cellular protection.

The final part of this PhD project pursued the study of the CLIC proteins' glutathionylation activity. Given their close resemblance to the GST-omega-1 proteins, we hypothesised that members of the CLIC family would like them, be capable of deglutathionylation activity. Using a synthetic peptide substrate, our *in vitro* studies demonstrate members of the CLIC family have significant deglutathionylation activity supporting a major role for them in the cellular glutathionylation cycle.

In conclusion, our results provided new insights into the functions of the CLIC proteins as soluble enzymes, with functions in cellular antioxidant protective mechanisms. Most importantly, it points to their role as post-translational regulators of target proteins via their deglutathionylation activity.